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Transition metals and host-microbe interactions in the inflamed intestine

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Abstract

Host-associated microbial communities provide critical functions for their host. Transition metals are essential for both the mammalian host and the majority of commensal bacteria. As such, access to transition metals is an important component of host-microbe interactions in the gastrointestinal tract. In mammals, transition metal ions are often sequestered by metal binding proteins to limit microbial access under homeostatic conditions. In response to invading pathogens, the mammalian host further decreases availability of these micronutrients by regulating their trafficking or releasing high-affinity metal chelating proteins, a process termed nutritional immunity. Bacterial pathogens have evolved several mechanisms to subvert nutritional immunity. Here, we provide an overview on how metal ion availability shapes host-microbe interactions in the gut with a particular focus on intestinal inflammatory diseases.

Introduction

The intestinal microbiota serves critical functions to human body, ranging from aid of food digestion, providing critical nutrient, education and modulation of the immune system, and colonization resistance against invading pathogens. Perturbations such as antibiotic treatment and enteric pathogen overgrowth drive the gut microbiota into an imbalanced state termed dysbiosis. Microbiota dysbiosis is frequently associated with and may contribute to the exacerbation of disorders involving intestinal inflammation, such as infectious colitis and inflammatory bowel disease (IBD) (reviewed in (Pham and Lawley 2014; Tamboli et al. 2004; Winter and Baumler 2014)).

Nutrient availability in the large intestine is one key determinant of gut microbiota function and composition. With small metabolites being absorbed in the small intestine, the main carbon source for primary fermenters in the large intestine is dietary fiber, a mixture of complex polysaccharides. As such, changes in the macronutrient component of the diet have a profound impact on the microbiota composition (David et al. 2014). In addition to macronutrients, the diet contains micronutrients such as vitamins and the biologically relevant transition metals iron, manganese, molybdenum and zinc. These metal ions exhibit a versatile redox chemistry and therefore catalyze a wide range of reactions such as DNA replication, oxidative stress detoxification, respiration and energy generation. These

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properties render these metal ions essential for the host and gut microbes. Dietary micronutrient oversupply or limitation impact the composition and function of the gut microbiota on a broad scale. For example, infants receiving an iron fortified diet exhibit a decreased abundance of bifidobacterium and lactobacillus strains in their fecal microbiota and an increased risk for enteric infections (Jaeggi et al. 2015; Simonyte Sjodin et al. 2018).

To limit undesirable effects from their redox chemistry such as radical formation in the Fenton reaction, metal ions are bound by specific proteins during uptake, export, transfer, storage and recycling. During infection, inflammatory host responses further limit microbial access to metal ions. Metal trafficking and storage processes are modified and high-affinity, metal chelating proteins such as lactoferrin and calprotectin are released during inflammation. These ion withholding strategies are collectively termed nutritional immunity (recently reviewed in (Hood and Skaar 2012; Lopez and Skaar 2018)). Although most of the mechanisms involved in nutritional immunity were initially characterized in systemic sites where the maintenance of sterility is crucial, similar principles likely apply to the gastrointestinal track (Zackular et al. 2015). Yet, metal withholding in the intestinal tract is likely to impact both the pathogenic and the commensal bacteria. Of note, the presence of the metal-chelating proteins lactoferrin and calprotectin in the feces are commonly used as clinical markers of gut inflammation (Walsham and Sherwood 2016). Bacterial pathogens have evolved sophisticated mechanisms to subvert host nutritional immunity, but little is known about how commensal gut bacteria acquire transition metals. In this review, we will focus on how competition for iron, manganese, molybdenum, and zinc shapes host-microbe interactions in the inflamed intestine. In addition, we will use *Salmonella* to illustrate how nutritional immunity in the inflamed intestine can be overcome by enteric pathogens.

Mammalian iron metabolism during homeostasis and inflammatory diseases

In mammals, iron is predominantly derived from the diet. Under physiological condition, iron exists in either reduced ferrous (Fe^{2+}) or oxidized ferric state (Fe^{3+}). Due to food processing techniques and the presence of oxygen in the stomach, dietary iron enters the small intestine predominantly in the form of ferric iron (Jacobs and Miles 1969). Uptake of dietary iron mainly occurs in the duodenum (Fig. 1A). Dietary iron can be grouped into two categories, heme iron and non-heme iron. Heme is taken up through the action of heme carrier protein 1 (HCP1) located on the brush-border membrane of duodenal enterocytes (Shayeghi et al. 2005) and subsequently degraded by heme oxygenase to yield ferrous iron intracellularly (Raffin et al. 1974). Non-heme ferric iron is reduced to the ferrous state by the cytochrome b reductase 1 (Dcytb) located in the brush-border membrane (McKie et al. 2001) and taken up through the divalent metal transporter 1 (DMT1) on the apical membrane of enterocytes (Gunshin et al. 1997). After uptake, ferrous iron can either be oxidized to ferric state by the ferroxidase activity of the iron storage protein ferritin and subsequently stored in ferritin (Lawson et al. 1991). Alternatively, iron is transported across the basolateral membrane by ferroportin into the plasma (Donovan et al. 2000; McKie et al. 2000). Plasma ferrous iron is then re-oxidized by the multicopper oxidase hephaestin (Griffiths et al. 2005), loaded onto the iron-binding glycoprotein transferrin and delivered to target tissue. In the body, most of the iron is bound by heme coordinated hemoglobin in the red blood cells. During events such as hemolysis, hemopexin and haptoglobin capture free

heme and hemoglobin respectively to minimize free iron level in plasma (Alayash et al. 2013; Delanghe and Langlois 2001). Therefore, during iron absorption, export, transfer, storage and recycle, the concentration of free iron is maintained at low level (Fig. 1A).

During episodes of inflammation, the host limits microbial access of iron. This process is in part controlled by the hormone hepcidin. Hepcidin controls iron concentration in extracellular fluid and blood plasma by regulating ferroportin-mediated iron export (McKie et al. 2000) (Fig. 1B). Hepcidin production is greatly enhanced in inflammatory diseases such as IBD and bacterial infections. By promoting the internalization and degradation of ferroportin, hepcidin reduces dietary iron absorption in the small intestine and the subsequent export into circulation, leading to hepcidin-induced-hypoferremia (HIF) (Nicolas et al. 2002). HIF is proposed as a defense mechanism against bacterial pathogens at systemic sites by limiting free iron in extracellular milieu and the circulation (Michels et al. 2015). For example, HIF is critical for controlling *Vibrio vulnificus* in a subcutaneous infection model (Arezes et al. 2015). However, how hepcidin influences enteric pathogen infection in the intestinal track remains unclear. Results from clinical studies on hepcidin levels and the severity of intestinal inflammation have not been consistent (reviewed in (Cherayil et al. 2011)). Infection of mice with *Salmonella enterica* serovar Typhimurium (S. Tm), a common cause of foodborne illness, induces hepcidin transcription. Interestingly, pharmaceutical inhibition of hepcidin transcription ameliorated *Salmonella*-induced and non-infectious colitis (Wang et al. 2009; Wang et al. 2012).

In addition to regulating iron trafficking, mammals also produce high-affinity iron binding proteins such as lactoferrin to sequester iron from microbes. Lactoferrin is a glycoprotein that exerts anti-microbial effects on a wide-range of bacterial species. Its bacteriostatic function is largely dependent upon its ability to chelate iron (Valenti and Antonini 2005). Lactoferrin is present in most mucosal surface throughout the body and its expression is induced by inflammation. In the lumen of the inflamed intestine, transmigrated neutrophils produce and release lactoferrin, which accumulates to levels exceeding 200 g/g under inflammatory conditions, while in control patients, lactoferrin levels are scarce (0.0–2.1 g/g) (Sipponen et al. 2008). The exact contribution of lactoferrin to generating an iron-limiting environment in the inflamed gut lumen remains to be shown experimentally. Curiously, human and bovine lactoferrin enhances growth of *Bifidobacterium* spp. *in vitro* (Petschow and Talbott 1991) and in piglets (Tang et al. 2009). Whether this “bifidogenic” effect is due to iron metabolism or degradation of apo-lactoferrin, a glycosylated peptide, is somewhat unclear (reviewed in (Oda et al. 2014)).

Bacterial iron acquisition

Gut bacteria encode a plethora of systems to acquire iron in the form of ferrous iron, ferric iron, heme, hemoglobin, transferrin, and lactoferrin (Fig. 1B). Specific examples of iron acquisition pathways in gut microbes are listed in Table 1. Iron acquisition by Enterobacteriaceae family members, such as *E. coli* and *S. Tm*, has been extensively studied *in vitro* and *in vivo* (reviewed in (Braun 2003)). Uptake of ferrous iron in *E. coli* is mediated by the FeoABC system (Kammler et al. 1993). Mutants defective in *feoABC* uptake are impaired for murine gut colonization (Pi et al. 2012; Stojiljkovic et al. 1993).

Siderophores, which are low molecular weight, high-affinity iron chelators, are produced when iron becomes limited. Examples of siderophores and their cognate uptake systems in common gut bacteria are listed in Table 1. Analysis of bacterial transcription profile in the murine intestinal tract revealed that *E. coli* in the colonic mucus layer express genes necessary siderophore biosynthesis and transport, suggesting that ferric iron is present in the lumen of the colon but its availability may be limited (Li et al. 2015).

Siderophores allow bacteria to scavenge iron from host chelators, such as transferrin and lactoferrin (reviewed in (Caza and Kronstad 2013)). *E. coli* and many other Gram-negative bacteria produce the catecholate siderophore enterobactin. Utilization of siderophores such as enterobactin (enterochelin) typically involves a TonB-dependent outer membrane receptor (e.g. FepA) which recognizes and translocates iron-laden siderophores (e.g. ferric enterobactin) into the periplasmic space using the energy transduced by the TonB-ExbB-ExbD system (Frost and Rosenberg 1975; Pugsley and Reeves 1976; Skare et al. 1993). In the periplasm, a periplasmic binding protein (e.g. FepB) relays the siderophore to an ABC-transporter imbedded in the inner membrane (e.g. FepCDG), which transports the siderophore into the cytoplasm.

Due to the lack of an outer membrane, Gram-positive bacteria possess a relatively simple siderophore transport system. These systems are typically composed of a siderophore-binding protein and a cognate permease embedded in the cell membrane (Wilson et al. 2016). Members of the human microbiota belonging to the phylum Firmicutes, such as *Staphylococcus* spp. and *Bacillus* spp. possess such siderophore transport system (Beasley et al. 2009; Miethke et al. 2006) (Table 1).

In both Gram-negative and Gram-positive bacteria, siderophore-bound iron needs to be released in the cytoplasm. Iron can be freed from siderophores by esterases that hydrolyze the siderophore backbone. For instance, Fes and IroD encoded by members of Enterobacteriaceae cleave iron-laden enterobactin and salmochelin respectively, into monomers or multimers of dihydroxybenzoate (DHBS). DHBS is a siderophore itself and can be re-secreted outside of the bacteria cells by specific efflux pumps like EntS and IroC (Caza et al. 2011). Alternatively, because ferrous iron has a significantly lower affinity for siderophores, some bacterial species employ ferric siderophore reductases to reduce ferric iron to ferrous state, to facilitate iron dissociation from siderophores (Schroder et al. 2003).

Pathogenic bacteria produce a wide range of siderophores to circumvent nutritional immunity and facilitate host colonization. Akin to *E. coli*, *S. Typhimurium* produces enterobactin and the cognate uptake system (FepABCDG). The affinity of enterobactin for iron is high ($K_d = 10^{-52}$), allowing *S. Typhimurium* to compete with host proteins such as lactoferrin ($K_d = 10^{-20}$) for iron (Baker and Baker 2004). Mutants deficient for the outer membrane receptor FepA are attenuated for colonization of systemic sites in a mouse model of disseminated Salmonellosis (Nagy et al. 2013). During infection, neutrophils and epithelial cells produces the protein lipocalin-2, which sequesters iron-laden enterobactin (Flo et al. 2004; Goetz et al. 2002). To subvert host nutritional immunity, *S. Typhimurium* produces salmochelin, a “stealth” siderophore which incorporates two additional glucosyl moiety to the enterobactin backbone (Hantke et al. 2003). Salmochelin is not recognized by lipocalin (Fischbach et al.

2006) and thus allows *S. Typhimurium* to effectively escape nutritional immunity conferred by both lactoferrin and lipocalin (Fig. 1C). In mouse models, mutants lacking the receptor for Salmochelin uptake (IroN) are impaired for colonization of systemic sites and do not thrive in the inflamed gut lumen (Costa et al. 2016; Nagy et al. 2013; Raffatellu et al. 2009). Most of the commensal Enterobacteriaceae members produce enterobactin but not salmochelin. Therefore, inflammation dependent production of lipocalin-2 suppresses commensal Enterobacteriaceae, which contributes to colonization resistance against *S. Typhimurium* by competing for common nutrition resources (Kamada et al. 2013). By overcoming iron starvation mediated by lipocalin-2, *S. Typhimurium* sidesteps the competition with the commensal Enterobacteriaceae (Behnsen et al. 2014; Raffatellu et al. 2009) (Fig. 1C).

Interestingly, the *E. coli* Nissle 1917 strain produces and utilizes at least four distinct siderophores, enterobactin, salmochelin, aerobactin, and yersiniabactin (Patzner et al. 2003). Administration of Nissle 1917 as a probiotic intervention during murine *S. Typhimurium* infection reduces pathogen colonization and fecal shedding. The ability to displace *S. Typhimurium* was dependent on siderophore uptake systems in Nissle 1917, suggesting that competition for micronutrients is an important aspect of gut colonization during episodes of inflammation (Deriu et al. 2013).

Iron is also essential to bacteria belonging to other phyla such as Bacteroidetes and Firmicutes, the predominant phyla in the gut microbiome (Table 1). During homeostasis, unabsorbed dietary iron available in the colon should be sufficient to support the growth of commensal bacteria. Under this condition, commensals such as Bacteroidetes and Firmicutes can acquire iron from heme, hemoglobin or by direct uptake of inorganic iron (Ho and Ellermeier 2015; Mazmanian et al. 2003; Rocha et al. 2018). However, these forms of iron are less microbially accessible during intestinal inflammation, as a *E. coli* mutant strain defective in siderophore utilization is severely attenuated in colonizing the inflamed gut. Interestingly, Bacteroidetes, do not appear to produce known siderophores (Otto et al. 1988; Rocha et al. 1991). Two recent *in vitro* studies demonstrated that some Bacteroides species are capable of utilizing siderophores that are produced by other bacteria or fungi (xenosiderophores), a phenomenon known as siderophore piracy (Moschen et al. 2016; Rocha and Krykunivsky 2017) (Fig. 1B). Conversely, *Aspergillus* strains can utilize bacteria-derived enterobactin through a specific siderophore transporter (Haas et al. 2003). Siderophore piracy also occurs during infection with enteric pathogens. For instance, *S. Typhimurium* encodes FhuA, a TonB-dependent outer membrane receptor to transport fungal siderophore ferrichrome (Killmann et al. 1998). However, the mechanisms underlying Bacteroides xenosiderophore utilization and whether siderophore piracy allows Bacteroides to overcome iron limitation *in vivo* remain to be explored.

Manganese

Manganese homeostasis in human health and disease

Manganese is essential for many cellular activities, as it functions as a cofactor for enzymes including arginase, glutamine synthetase, pyruvate carboxylase and manganese superoxide dismutase. Diet is the main source of manganese, and approximately 5% of the dietary

manganese is absorbed in the gastrointestinal tract. DMT1, the divalent metal transporter responsible for uptake of the majority of dietary iron, is considered to be the major transporter for manganese influx (Illing et al. 2012) (Fig. 2A). Biochemical evidence suggests that manganese could be stored in ferritin (Ardini et al. 2018). Intracellular manganese is exported via ferroportin (Yin et al. 2010) and bound by transferrin for delivery to destination tissues (Davidsson et al. 1989). Manganese in the body is bound by gamma-globulin, albumin and transferrin (Aisen et al. 1969).

Many molecular mechanisms involved in manganese uptake and transport are shared with iron, which ensures manganese in the body is tightly sequestered from microbial use. During inflammation, the host can further sequester manganese in several ways. Phagocytic cells such as macrophages and neutrophils engulf bacteria and isolate them in phagosomal compartments, where manganese is depleted through the action of Natural Resistance-Associated Macrophage Protein 1 (NRAMP1) and related divalent metal transporters such as NRAMP2 (Jabado et al. 2000). Neutrophils can also limit bacterial access to manganese by producing lactoferrin and calprotectin, high affinity iron/manganese and manganese/zinc binding proteins respectively (Corbin et al. 2008). Calprotectin is member of the S100 protein family and a heterodimer of S100A8 and S100A9, each of which contains two EF-hand Ca^{2+} binding sites. The dimer interface forms two zinc binding sites and one manganese binding site (Damo et al. 2013). High level of calprotectin released by neutrophils during gut inflammation (125–250 g/ml) (Liu et al. 2012) contributes to manganese limitation in the inflamed intestine.

Bacterial manganese acquisition and virulence

During intestinal inflammation, reactive oxygen species (ROS) are released by inflammatory cells as an antimicrobial defense mechanism (Aviello and Knaus 2017). Bacterial defense against host oxidative stress is highly dependent on manganese. Manganese can efficiently catalyze superoxide disproportionation in the form of low molecular weight manganese complexes such as manganese phosphate and manganese carbonate (Barnese et al. 2008; Barnese et al. 2012), or serve as a cofactor in ROS detoxification enzymes such as manganese superoxide dismutase (Mn-SOD) and manganese-containing catalase. Manganese dependent, SOD-independent superoxide detoxification has been reported in *Staphylococcus aureus* and certain *Lactobacillus* strains (Archibald 1986; Kehl-Fie et al. 2011). Since some *Lactobacillus* strains lack SOD, they may depend on molecular weight manganese complexes to detoxify superoxide.

ROS-detoxifying enzymes that use manganese contribute to the virulence of enteric bacterial pathogens (Diaz-Ochoa et al. 2016; Wan et al. 2017). Enteric pathogens such *S. Typhimurium* possesses multiple manganese transporters (SitABCD, MntH and ZupT) to overcome calprotectin-mediated sequestration of manganese (Fig. 2B). Effective acquisition of manganese ensures proper functions of catalase and SOD, enabling *S. Typhimurium* to resist neutrophil killing and outcompete the commensals and thrive in the inflamed intestine (Diaz-Ochoa et al. 2016). Manganese acquisition is also important for the pathogenesis of *Enterococcus faecalis*, a member of the gut flora and a leading cause of nosocomial systemic infections (Ubeda et al. 2010). The ability to acquire manganese using high-affinity transport

system is essential for *E. faecalis* virulence during systemic spread (Colomer-Winter et al. 2018).

In addition to defending against host-derived ROS, manganese could contribute to detoxification of superoxide produced during bacterial respiration. Depletion of butyrate-producing obligate anaerobes, a scenario frequently associated with antibiotic treatment or intestinal inflammation, drives metabolic switch from beta-oxidation to glycolysis in the gut epithelium (Donohoe et al. 2011; Donohoe et al. 2012). Switch in metabolic program in the gut epithelial cells leads to increased availability of oxygen in the gut lumen (Byndloss et al. 2017; Kelly et al. 2015). Under this circumstance, it is conceivable that *Lactobacillus* can use oxygen as the terminal electron acceptor and produce large quantity of hydrogen peroxide, with superoxide as the byproduct. Experimental evidence suggested that some *Lactobacillus* do produce hydrogen peroxide in the inflamed gut or in host NADPH oxidase deficiency (Archibald and Fridovich 1981; Pircalabioru et al. 2016; van Niel et al. 2002). It is conceivable that manganese -dependent enzymes such as Mn-SOD (Sanders et al. 1995) may be important for a respiratory metabolism of *Lactobacillus* in intestinal lumen.

Zinc

Zinc homeostasis in human health and disease

Homeostasis of zinc in humans is mostly maintained by absorption of dietary zinc in the gastrointestinal track, secretion and transport of endogenous zinc from the gastrointestinal system to target organs. Zinc deficiency represents significant clinical burden worldwide. It is known that dietary factors have a strong impact on zinc absorption by influencing the amount of zinc in the small intestine lumen (Gibson 1994a; Gibson 1994b). In this regard, zinc deficiency is most common in areas where animal-sourced food ingestion is low while plant consumption is high. It is important to note that the bioavailability of this metal in the gut rather than diet has direct impact on zinc deficiency. For instance, phytic acids, which are present in seeds and grains, bind zinc with high affinity. Phytic acid bound cannot be absorbed in the gut lumen (Hambidge et al. 2011). Zinc becomes available in the gut lumen postprandially and most of the zinc is absorbed in the small intestine (Cousins 1985; Krebs 1998; Lee et al. 1989; Matseshe et al. 1980) (Fig. 2A). After uptake, zinc is transported into the enterocytes in the duodenum and jejunum, translocated from apical to basolateral membrane then into the portal circulation (Reyes 1996). Zinc transport in mammalian cells is mediated by two protein families. The first class of proteins are mainly zinc importers (Krebs 2000). These proteins are known as Zip (Zrt- and Irt-like proteins (SLC39A)), which promote zinc transport from extracellular fluid or from intracellular vesicles into the cytoplasm (McMahon and Cousins 1998a; McMahon and Cousins 1998b). In the Zip family, Zip4, which is localized at the apical membrane of the enterocytes, is the major transporter responsible for uptake of dietary zinc (Wang and Zhou 2010). The second class of proteins involved in zinc transport is ZnT (solute-linked carrier 30 (SLC30A)), and their functions are to mediate zinc efflux from cells or influx into intracellular vesicles under the condition of zinc excess. The mammalian ZnT family consists of 10 members (ZnT1–10) (Cousins et al. 2006). The expression of the ZnTs is mostly regulated by changes in zinc concentration. These proteins are predominantly found in intracellular compartments, such as Golgi or

endoplasmic reticulum, except for ZnT1 which is located on the plasma membrane. ZnT1 is expressed in the villi of the proximal small intestine, and studies in rats showed that expression of ZnT1 is increased in response to zinc supplementation through diet (McMahon and Cousins 1998a; McMahon and Cousins 1998b), consistent with the idea that main role of ZnT1 is to regulate cellular zinc efflux (Palmiter and Findley 1995). These evidence also lead to the proposition that ZnT1 may be responsible for transporting dietary zinc from the basolateral side of the polarized enterocytes into circulation (Wang and Zhou 2010).

In addition to ZnTs and Zips, metallothionein also regulates zinc absorption (Cousins et al. 2006). Metallothioneins are intracellular metal binding proteins that are highly expressed in parenchymal cells of the intestine, liver, kidney and pancreas. Metallothionein expression is positively correlated to zinc availability, and serum zinc concentration was inversely related to metallothionein level. Davis *et al.* found that after dietary zinc supplementation, serum zinc concentration was higher in metallothionein-deficient mice compared to control animals. These data suggested that metallothionein serves as a zinc pool in case of elevated zinc intake (Davis et al. 1998).

Bacterial zinc acquisition and virulence

In the human body zinc is primarily distributed in the skeletal muscle and bone, but is also found in other organs such as spleen, liver and kidneys. Only a small percentage of the zinc is present in the blood, which is mostly bound to albumin (Wastney et al. 1986). In the tissue, nutritional immunity is imposed by mammalian zinc transporters or high-affinity zinc binding proteins. During acute phase of inflammation, IL-6 mediated upregulation of Zip8 and mobilization Zip14 to the sinusoidal membrane of hepatocyte contribute to the increased zinc uptake from plasma (Liu et al. 2013; Liuzzi et al. 2005). In addition, NRAMP family members function as general metal ion transporters and can sequester zinc from intracellular pathogens. IL-6 expression also induces expression of zinc-sequestering protein metallothionein (Gaetke et al. 1997). These mechanisms contribute to systemic hypozincemia as a component of acute phase response (Gabay and Kushner 1999). In the gut, metallothionein is expressed in the colonic submucosa in murine colitis models. Importantly, genetic ablation of metallothionein is associated with increased expression of inflammatory markers such as TNF-, suggesting an anti-inflammatory role of metallothionein (Al-Gindan et al. 2009; Tsuji et al. 2013). However, whether metallothionein-mediated alleviation of inflammation depends on zinc chelation remains to be determined.

Calprotectin is a key component to withholding zinc during bacterial infection in systemic sites and in the intestine (Corbin et al. 2008; Kehl-Fie and Skaar 2010; Liu et al. 2012). For instance, calprotectin inhibits *Staphylococcus aureus* growth in abscesses, and its chelation virtually renders the abscesses free of zinc (Corbin et al. 2008). Calprotectin makes up to 60% of the cytoplasmic proteins in neutrophils (Gebhardt et al. 2006) and its expression can be further induced by IL-22 and IL-17 during intestinal inflammation (Hood and Skaar 2012). Neutrophil transmigration is an important characteristic of immune responses during infectious and non-infectious colitis (Day et al. 1978; Zhou et al. 2018), and the resulting release of calprotectin can reach up to 250 g/ml in the fecal content, making it a sensitive

biomarker for intestinal inflammation. Together, these data suggested that the inflamed intestine is a zinc limiting environment and calprotectin is an important contributing factor.

Zinc uptake in bacteria is regulated by Zur, zinc uptake regulator, which regulates intracellular zinc levels by controlling zinc uptake. Zur represses transcription of gene associated with zinc import when zinc is abundant, and derepresses its regulon in the absence of zinc (Outten et al. 2001). Bacterial pathogens such as *S. Typhimurium*, *Brucella abortus*, *Listeria monocytogenes*, *E. coli* and *Yersinia pestis* (Ammendola et al. 2007; Corbett et al. 2012; Desrosiers et al. 2010) express high-affinity zinc transporters homologous to the ZnuABC system in *E. coli* (Fig 2B). This system belongs to the ATP-binding cassette transporters family and is composed of three proteins: ZnuA, ZnuB, ZnuC, which are a soluble periplasmic metallochaperone, a membrane permease, and an ATPase receptively (Patzner and Hantke 1998). In the inflamed intestine, lipocalin-2 and calprotectin suppress the growth of commensal bacteria through metal limitation (Valeri and Raffatellu 2016).

Zinc availability and bacterial access to zinc in the gut influences enteric pathogen infection. The ZnuABC system allows *S. Typhimurium* to subvert host nutritional immunity to outcompete commensal bacteria and thrive in the inflamed gut (Behnsen et al. 2014; Liu et al. 2012). *Clostridium difficile* is a Gram-positive pathogen that infects the large intestine and causes a wide spectrum of diseases varying from mild diarrhea to pseudomembranous colitis, collectively called *Clostridium difficile* colitis (CDC) (Lessa et al. 2015). One of the most important risk factors for CDC is antibiotics use. In mice, antibiotic perturbation of the microbiota is required for *C. difficile* to establish infection and cause disease. In a recent report, Zackular *et al.* showed that excess dietary zinc introduced significant alterations to the composition of the microbiota, characterized by reduced alpha diversity and outgrowth of certain *Enterococcus* and *Clostridium* members. More importantly, excess zinc significantly lowered the threshold of antibiotic perturbation required for *C. difficile* infection. Detailed investigation into the dynamic of the microbiota structure associated showed that mice fed with normal zinc diet quickly recovered to baseline post low-dose antibiotic treatment while the mice fed with excess zinc failed to do so (Zackular et al. 2016; Zackular and Skaar 2018). This is likely to be the cause of the increase susceptibility to *C. difficile* infection, as a normal microbiota confers robust colonization resistance against *C. difficile* colonization (Buffie et al. 2015). These findings highlight the importance of zinc as a determinant of host-microbiome-pathogen integrations.

Molybdenum

Molybdenum homeostasis in health and disease

Molybdenum is a transition metal found in nearly all biological systems. Its capability to shuttle from between the +4/+5 and the +5/+6 redox states allows molybdenum-containing enzymes to catalyze reactions of a wide range of redox potentials under physiological conditions (Hille 2002; Mendel and Bittner 2006). Molybdenum can be taken up from diet and excreted in urine (Turnlund et al. 1995). Molybdenum is taken up in the form of the oxyanion. Two specific molybdate transporters identified in eukaryotes are the MOT1 and MOT2 from the alga *Chlamydomonas reinhardtii*. Homologues of MOT2 have been detected

in mammals including humans (Tejada-Jimenez et al. 2011), however, molybdenum uptake and transport in mammalian cells is incompletely understood.

In prokaryotic and eukaryotic cells, molybdenum is only biologically active when it is properly coordinated by a organophosphate-dithiolate scaffold, named molybdopterin, to produce the molybdenum cofactor (Mo-co) (Hille 2002). Based on the type of pterin derivative coordinating the molybdenum atom, molybdoenzymes can be classified into three families: the xanthine oxidase (XO) family, the sulfite oxidase (SO) family, and the dimethyl sulfoxide (DMSO) reductase family (Hille et al. 2011). Members of these families utilize sulfurated MPT-cytosine dinucleotide (MCD), di-oxo Mo-co, and bis-MPT-guanine dinucleotide (bis-MGD) as their cofactors, respectively. Seven molybdenum enzymes have been identified in eukaryotes, which are sulfide oxidases, aldehyde oxidases, xanthine dehydrogenases, and mitochondria amidoxime reducing component (mARC) (Mendel and Kruse 2012). The importance of molybdenum to mammalian hosts is evidenced by the fact that Mo-co deficiency results in rare but severe inborn error of metabolism (reviewed in (Schwarz 2016)). However, how the bioavailability of molybdenum is regulated in mammalian hosts is not well understood, and no immune defense mechanism dedicated to sequestering molybdenum have been identified so far.

Molybdenum cofactor in host-microbe interactions in the gut

In bacteria, molybdate is acquired by a high-affinity ABC-type transporter ($K_d \sim 50$ nM), or two other promiscuous anion transporters (Self et al. 2001). Inside the cell, molybdenum is incorporated into the Mo-co in a highly conserved, multi-step process (reviewed in (Magalon and Mendel 2015)). Briefly, the biosynthesis of Mo-co in bacteria involves the synthesis of molybdopterin, incorporation of molybdenum to form Mo-co and the subsequent modifications to form Mo-co variants such as bis-MGD. The maturation of Mo-co is then followed by the insertion of Mo-co deep inside of the target enzymes in a molecular chaperon dependent manner (Genest et al. 2009).

In bacteria, molybdoenzymes catalyze a wide range of reactions related to the electron transport chain. Respiratory and fermentative formate dehydrogenases, nitrate reductases, and other anaerobic reductases are Mo-co-dependent. Among the gut bacterial phyla, Proteobacteria encode the highest amount of molybdoenzymes (Rothery and Weiner 2015). Proteobacteria are normally a minor ($\sim 0.1\%$) constituent of the gut microbial community, but undergo significant expansion during episodes of intestinal inflammation. In the inflamed intestine, formate oxidation and nitrate respiration independently contribute to the bloom of Enterobacteriaceae (Proteobacteria phylum) (Hughes et al. 2017; Winter et al. 2013). Importantly, these pathways are largely specific to Enterobacteriaceae and are only operational during intestinal inflammation. Tungsten, a transition element closely related to molybdenum, can replace molybdenum in Mo-co when tungsten is present in excess (Aurich 1959). Due to the difference in redox properties, the tungsten-substituted Mo-co inhibits the activities of proteobacterial molybdoenzymes including nitrate reductases and formate dehydrogenases (Gates et al. 2003). In mouse models of colitis, oral administration of sodium tungstate abrogates the fitness advantage conferred by molybdoenzyme-dependent respiration in Enterobacteriaceae (Zhu et al. 2018).

Conclusions and remarks

Over the past decades, numerous seminal studies have greatly advanced our understandings in the importance of transition metal ions in host-microbe interactions. In recent years, the microbiota became the new focus of transition metal ion research because its intimate association with human health and diseases. The complex nature of the microbial ecosystem in the gut hampers the development of microbiome-targeted intervention strategies to improve human health. Transition metal ions represent an important starting point for the development of such strategies, as they are crucial for the host, commensal bacteria, and enteric pathogens. Dietary supplement of metal ions has strong influence on the microbiota structure and function, but the outcomes of different studies have not been consistent and are poorly predictable *a priori*. This is largely due to the lack of mechanistic understanding on how metal ions impact bacterial physiology in such a complex ecosystem. For instance, it is unclear how commensals acquire metals in the intestinal tract during intestinal inflammation. Future experimental work can use the principles of host-microbe metal competition gained by studying model enteric pathogens to characterize mechanisms involved in commensals coping with metal starvation. These insights could be exploited to uncover novel treatment strategies to ameliorate diseases and promote homeostasis.

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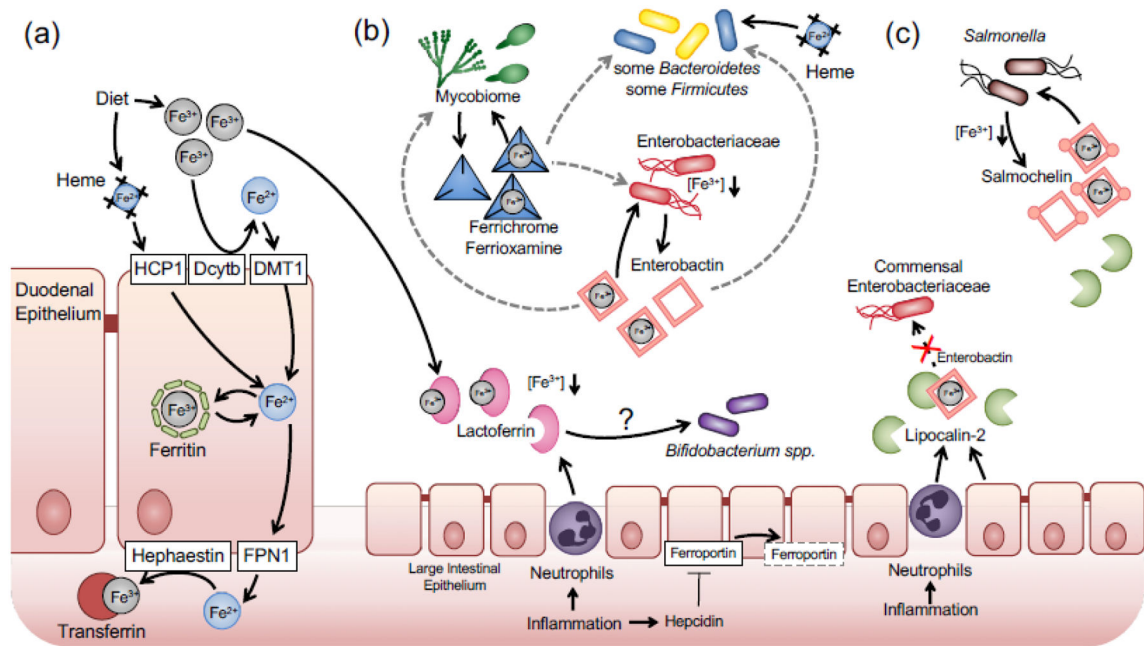


Fig. 1. Iron uptake by the host and gut-associated microbial communities during inflammation.

(a) Under homeostatic conditions. Duodenal cytochrome B (Dcytb) converts Ferric iron to ferrous iron, which is transported into duodenal epithelial cells by the Divalent Metal Transporter 1 (DMT1). Iron is either stored intracellularly in complex with ferritin or released at the basolateral side through ferroportin (FPN1). Hephaestin oxidizes ferrous to ferric iron, which is bound by transferrin in the serum, (b) During intestinal inflammation, lactoferrin is released into the gut lumen. Hepcidin mediates degradation of ferroportin and decreased iron uptake by the host. Bacterial communities acquire iron through a number of different mechanisms, including heme and siderophoras. For example, Enterobacteriaceae family members produce enterobactin during iron-limiting conditions. Iron-loaded enterobactin can be used by the original producer or possibly by other communities such as some Bacteroides strains or fungal members of the microbiota. a process termed iron piracy (gray arrows), (c) Enteric pathogens such as *Salmonella* produce several siderophores such as enterobactin and salmochelin to overcome host iron sequestration. Neutrophil-derived lipocalin-2 sequesters enterobactin. Salmochelin, a glucosylated enterobactin derivative, is not recognized by lipocalin-2 and facilitates iron uptake by *Salmonella* during inflammatory conditions.

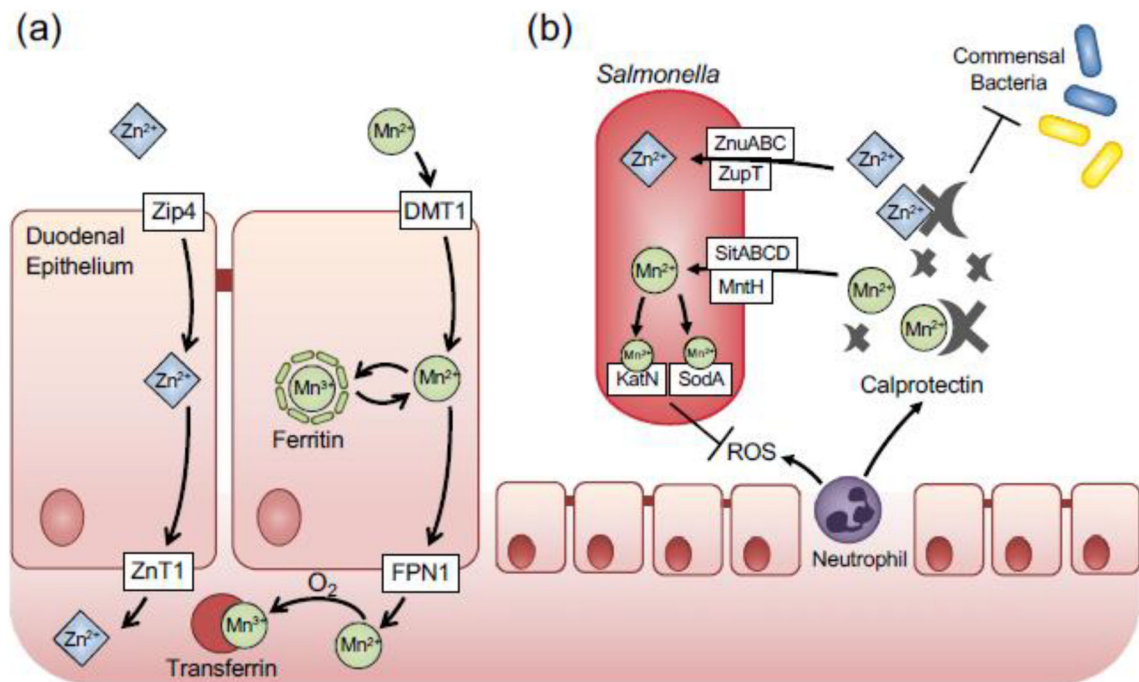


Fig. 2. Manganese and zinc homeostasis and subversion of nutritional immunity by *Salmonella Typhimurium*.

(a) Manganese and zinc uptake by the mammalian host in the duodenum. Zinc is imported into epithelial cells by Zrt- and Irt-like protein 4 (Zip4), and released on the basolateral side by Zinc Transporter 1 (ZnT1). Akin to iron, manganese is transported across the apical membrane of duodenal epithelial cells by the Divalent Metal Transporter 1 (DMT1), stored in ferritin, or exported on the basolateral side via ferroportin (FPN1). Free manganese is sequestered by transferrin and transported to target organs, (b) During intestinal inflammation, zinc and manganese are sequestered by calprotectin, which is released by epithelial cells and neutrophils. In addition, zinc-binding protein metallothionein is expressed in the colonic mucosa in the inflamed intestine. Calprotectin-mediated metal withholding suppresses growth of commensal bacteria. Enteric pathogen *Salmonella* uses several high-affinity transporters to subvert nutritional immunity and sidestep the competition with the commensal bacteria. Zinc-dependent superoxide dismutases and catalases help *Salmonella* defend against reactive oxygen species (ROS).

Table 1:

Examples of experimentally validated iron uptake systems in gut bacteria

Phylum	Iron source or siderophore	Transporter/receptor (in exemplary organisms)	Reference
Bacteroidetes	Heme	HupA	(Chen and Wolin 1981)
		FeoAB in conjunction with BtuS1 and BtuS2 (<i>Bacteroides fragilis</i>)	(Otto et al. 1996) (Rocha et al. 2018)
	Ferrous iron	FeoAB (<i>Bacteroides fragilis</i>)	(Rocha et al. 1991)
	Ferric iron	Unknown	(Rocha et al. 1991)
	Ferrichrome	FchA1/FchA2 (<i>B. fragilis</i>)	(Rocha and Krykunivsky 2017)
	Enterobactin	Unknown	(Rocha and Krykunivsky 2017)
	Salmochelin	Unknown	(Rocha and Krykunivsky 2017)
Firmicutes	Ferric citrate	YfmCDEF (<i>Bacillus subtilis</i>)	(Ollinger et al. 2006)
	Ferrous iron	FeoAB (<i>Clostridium perfringens</i>)	(Awad et al. 2016)
	Heme	ChtD/ChtE (<i>C. perfringens</i>)	(Choo et al. 2016)
	Hemoglobin	Unknown (<i>Prevotella intermedia</i>)	(Leung et al. 1998)
	Bacillibactin/Enterobactin	FeuA (<i>B. subtilis</i>)	(Peuckert et al. 2011)
	Ferrichrome	FhuDBGC (<i>B. subtilis</i>)	(Schneider and Hantke 1993)
	Ferrioxamine B	FoxD FhuBGC (<i>B. subtilis</i>)	(Schneider and Hantke 1993)
	Schizokinen	YfhA/YfiYZ (<i>B. subtilis</i>)	(Ollinger et al. 2006)
	Transferrin and lactoferrin	Unknown (<i>Enterococcus faecalis</i>)	(Lisiecki 2017)
	Ferric-2-oxoacid complex	Unknown (<i>E. faecalis</i>)	(Lisiecki and Mikucki 2006)
	Hydroxamate	Unknown (<i>Enterococcus</i> spp.)	(Sobis-Glinkowska et al. 2001)
Proteobacteria (Enterobacteriaceae)	Ferrous iron	FeoAB (<i>Escherichia coli</i>)	(Kammler et al. 1993)
	Ferric citrate	FecABCDE (<i>E. coli</i>)	(Wagegg and Braun 1981)
	Heme	ChuA (EHEC)	(Torres and Payne 1997)
	Enterobactin	FepABCDG (<i>S. Typhimurium</i>)	(Rutz et al. 1991)
	Yersiniabactin	FyuA (Yersinia, EHEC)	(Schubert et al. 1998)
	Salmochelin	IroN (<i>S. Typhimurium</i>)	(Baumler et al. 1998)
	Dihydroxybenzoate	CirA (<i>E. coli</i>)	(Cardelli and Konisky 1974; Hantke 1990)
		Fiu (<i>E. coli</i>)	
	Ferrichrome	FhuA (<i>S. Typhimurium</i>)	(Tsolis et al. 1995)
Bifidobacterium	Lactoferrin	Unknown	(Oda et al. 2014; Petschow and Talbott 1991)